Steven M. Ruben
Appl. No. 10/662,429

Department 3
Subject
Name
Address

National Brand
Computation Notebook
11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets 43-648

0 73333 43648 8

AVERY DENNISON
Office Products
Chicopee, MA 01022

Ruben EXHIBIT #129

Department 3

Subject

Name

Address

National Brand

Computation Notebook

11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets 43-648

0 73333 43648 8

AVERY DENNISON
Office Products
Chicopee, MA 01022

Ruben EXHIBIT 2129
Ruben v. Wiley et al.
Interference No. 105,077
RX 2129

2-8-96 Test OD for construct prior

4616 Trail HindIII 3'
4617 " BamH2 5''
4618 " BamH1 5'
4619 HT4 HindIII 3'
4620 " BamH1 5'
4621 HoFmBoJ BamH1 5'
4622 " Dsp718 3'

acemsamples Method Sequencer Print Vol

Results file: A:\WORK_RES Method name: A:\DEFAULT

Assay type: General Ratio and Concentration Units: ug/ml
Formula setup: VIEW Background Correction: (No)
Sampling device: One cell Concentration: (No)
Read average time: 0.50 sec Peak Pick: (No)

	Sample ID	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	1:500
1	4616	0.0515	0.0330	1.5821	0.6402	0.9
2	4617	0.0400	0.0278	1.4471	0.6910	0.7
3	4618	0.0394	0.0260	1.5126	0.6811	0.6
4	4619	0.0484	0.0326	1.4830	0.6743	0.8
5	4620	0.0445	0.0291	1.5301	0.6536	0.7
6	4621	0.0613	0.0374	1.6401	0.6097	1
7	4622	0.0550	0.0391	1.4065	0.7110	0.9
8						

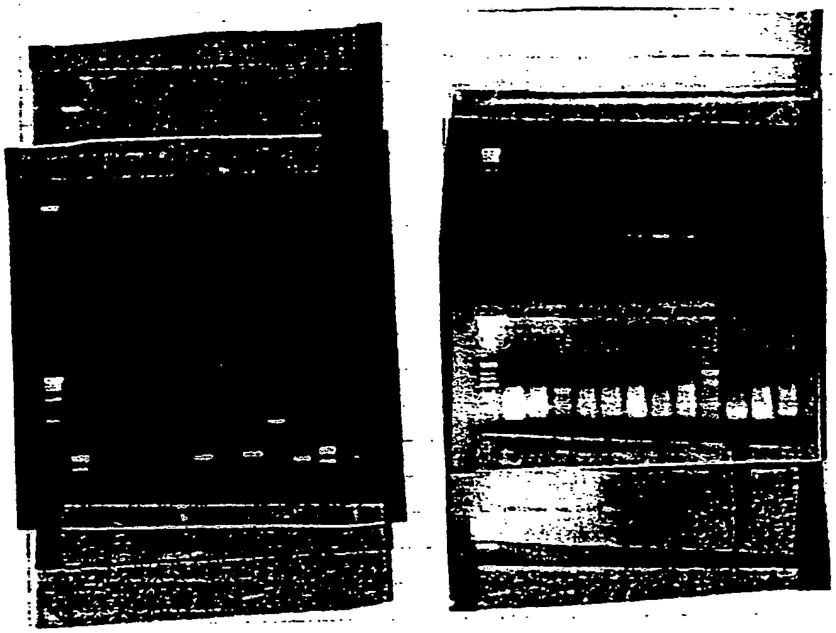
PCR for construct :

			Enzymes	Vector
1	HCAcub2	4319 + 4320 3'	BamH1 + Dsp718	pAz
2	HcAcub2	4319 + 14351 3'	" BamH1 "	cHa
3	HTpA (Trail)	4616 + 46175'	HindIII + BamH2	pQE9
4	HTpA (Trail)	4616 + 46185'	HindIII + BamH1	pQE9
5	HT4	4619 + 46205'	HindIII + BamH1	pQE9
6	HoFmBoJ	4621 + 46223'	BamH1 + Dsp718	pAz
7	HTTBV61	4577 + 4578 3'	BamH1 + xba1	Az-GP

2-8-96 PCR: for HITT BN61

use T3 primer + 4579 primer

Take 5μl Running 3% agarose gel for Southern Blot.



(1) HCQA
(2) HLGX
(3) HF
(4) HBm
(5) F. Brain
(6) Hela
(7) pinv leck
(8) HCE
(9) HET
(10) HBF
(11) HPL
(12) HTX
(13) HE8
(14) HCH
(15) HPD
(16) HSOB
(17) HIKI
(18) HKL
(19) HFS
(20) HHG
(21) LV A
(22) HHMA
(23) HTXA
(24) LV
(25) Hela

- Denature Buffer

labeling HITTBN61 BamHI specific primer → Buffer.

See oligo labeling

1λ	(1μg) HITTBN61 primer	
5λ	10x T4 PNK Buffer	at 30′
1λ	γ-32P ATP	
1λ	T4 Kinase Enzyme (PNK)	
42λ	H_2O	

37°C 30′ — primer BamHI

add 10μg of spinner DNA 100ml
5m NaPO4 PPT

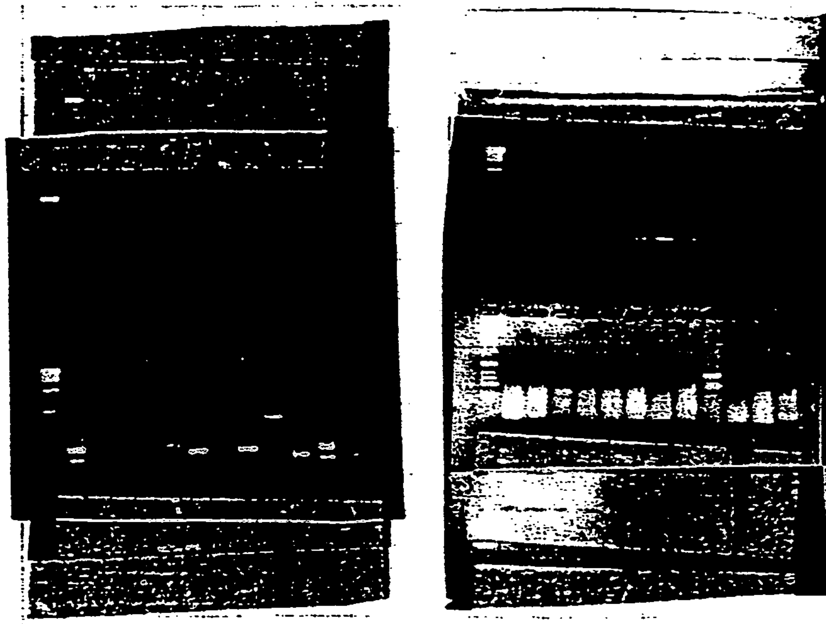
Counter: 1×10^8/200μl.

[signature] 2/9/96

2-8-96 PCR: for HTTBN61

use T3 primer + 4579 primer

Take 5ul Running 3% agarose gel for Southern Blot.



(1) HCQA
(2) HLGX
(3) HF
(4) HBM
(5) F. Brain
(6) Hela
(7) pInv-lac
(8) HCE
(9) HET
(10) HAF
(11) HPL
(12) HTX
(13) HE8
(14) HCH
(15) HPDI
(16) HSOB
(17) HIKI
(18) HKL
(19) HFS
(20) HMG
(21) LV
(22) HMMA
(23) HTXA
(24) LV
(25) Hela

- Denature Buffer
  Take the gel put in 300ml Denatures Buffer
  at RT 1 hr

= Neutralization Buffer
  300ml shake the gel at RT. about 30'

= at 10xSSC transfer o/weekend

- Make oligo probe: use HTTBN61 (4577) primer BamHI

Conter: 1×10^8 / 200ml.

[signature] 2/9/96

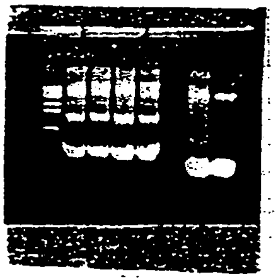
2-9-96 PCR for construct:

Running 2% low melting gel:

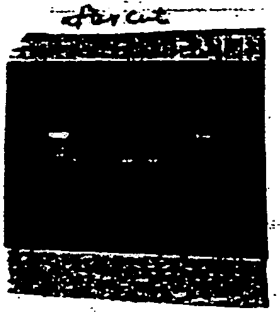
17) HIKI
18) HKL
19) HFS
20) HMG
21) HLWA
22) HHMA
23) HTXA
24) LV
25) Hzin

(NKEF)
1 HCAcub2
(NKEF)
2 HCAcub2
(Fas L)
3 HTPA

Before cut



after cut



(Fas L)
4 HTPA
(Fas Like)
5 HT4
6 HoFMB09
(TNFR)
7 HTTBN61

Before cut

after cut

at 70°C 10'
phenol / φ x3
ETOH ppt (o/weekend)

2-12-96 Degestion by Enzyme

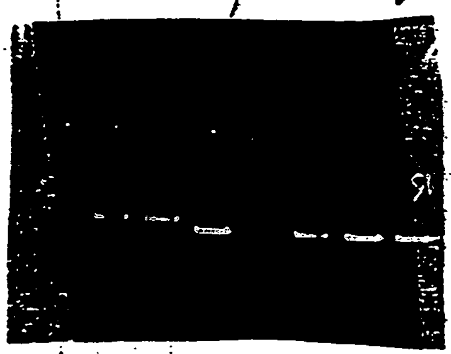
at 37°C incubate 2hrs

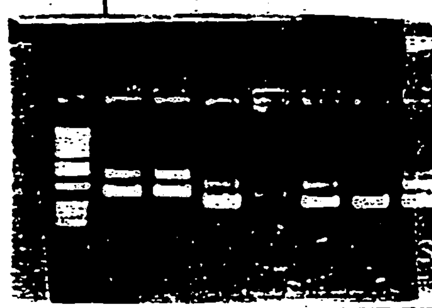
↓ phenol / φ x3

↓ ETOH ppt

30λ	
25λ	DNA + H2O
3λ	#2 Buffer
1λ	BamH1 Xba
1λ	HindⅢ Asp718

Take 3λ of each sample Running the gel





2-12-96 Submit CSG16 (CSG7) for sequencing

Hpp012

use pRE 3' + 5' primer

2-12-96 Ligation to vector.

#1	HCAQub2	pA2
2	"	cHO
3	HTPA	pRZ9
4	HTPA	pRZ9
5	HT4	pRZ9
6	HOFimBo9	pA2
7	HTTBub1	A2-GP

2-13-96 transformation to XL1Blue cell

2ml to 100ml XL1Blue cell

on ice 15'

42°C 1' add 1ml LB

37°C 1hr

pour plates 500 λ. (LB+Amp plate)

2-14-96 [redacted]:

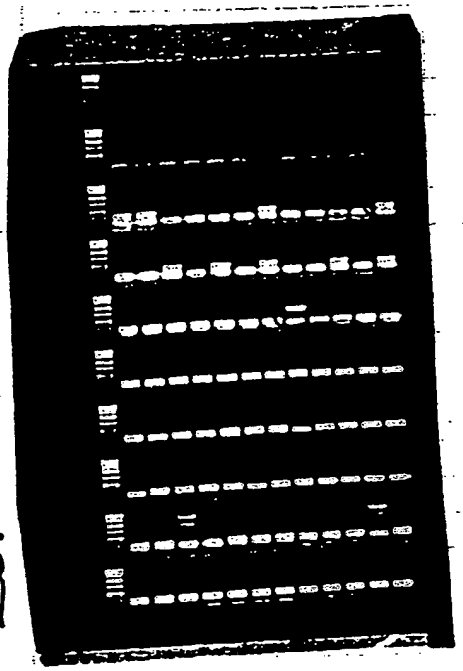
CSG

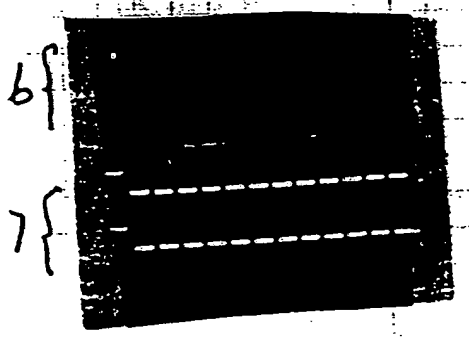
Wash Southern Blot: at RT 2 time

Exp. 4 hrs

2-14-96 pick up single clonesies To 100ml LB + Amp

make PCR Reaction:





grow up DNA

#2 — 12
#3 — 8
#7 — 1

2-15-96 all constructed PCR primer use vector primer

made DNA prep:

BECKMAN DU-600

Date:

Nucleic Acid ReadSamples	Method			
Hi Bomb				2.6
HTPA 3			1.5330	2.5
HTPA 7		1.0075	0.5532	2.5

2-14-96 pick up 12 clone from HTTBU61 PCR product

use Enlgland Buffer ppt.

-Clean: add 100ml Enlgland Buffer

at RT 10'

centrifuge 10'

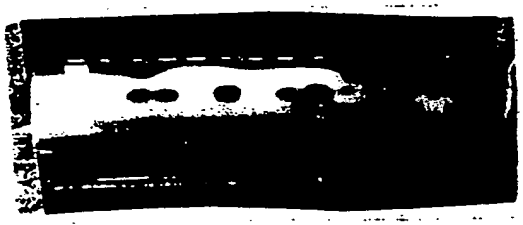
70% EtOH wash

Resuspened in 10ml of H_2O

Running on the gel to check the DNA

Submit for sequencing.

primer use HTTBU61 RPR3

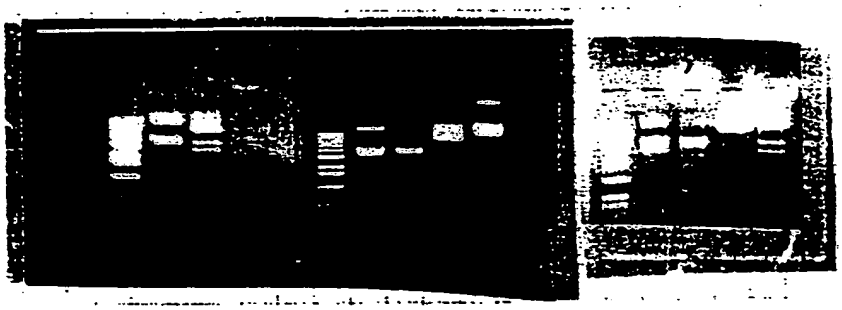


1 — 8 10 — 12 use 4ml of DNA

#9 use 1 ml of DNA.

2-15-96 Degestion:

1K	HCAEBU62 un	HCAEBU62 cut	HTPA un	HTPA cut	HTTBU61 un	HTTBU61 cut
	2		5		7	



2-15-96 ligation again

#1	HeAcub2	pA2
#3	HTPA (Trail)	pQE9
#4	HTPA (Trail)	pQE9
#5	HT4	pQE9
#6	HOFmB09	pA2
#7	HTTBN61	A2-GP

20λ

3λ	DNA insert
3λ	vector
4λ	5x Ligation-buffer
1λ	T4 DNA ligase
9λ	H2O

RT 3.5 hrs

2-16 PCR for construct : primer Enzymes Vector

(1)	HSABH13	4335 + 4336	[redacted]	pA2
(2)	HTXE133	4292 + 4293	[redacted]	pA2
(3)	HOFmB09	4621 + 4622	[redacted]	pA2
(4) ✓	HTTBN61	4577 + 4578	[redacted]	A2-GP
(5) ✓	HPDD012	4581 + TNFβ Hind3	[redacted]	pQE9
(6) ✓	HTPA (Trail)	4616 + 4617	[redacted]	pQE9
(7) (for sequencing number) (HTPAT) ←	HTPA (Trail)	4616 + 4654	[redacted]	pQE9
(8)	HT4	4619 + 4620	[redacted]	pQE9
(9)	FASL (HFASL6)	4657 + 4656	[redacted]	pQE9

2/20/96

2-20-96 Runing 2% low melting gel: (for construct)

cut down insert 70°C 10'

phenol/φ x-3

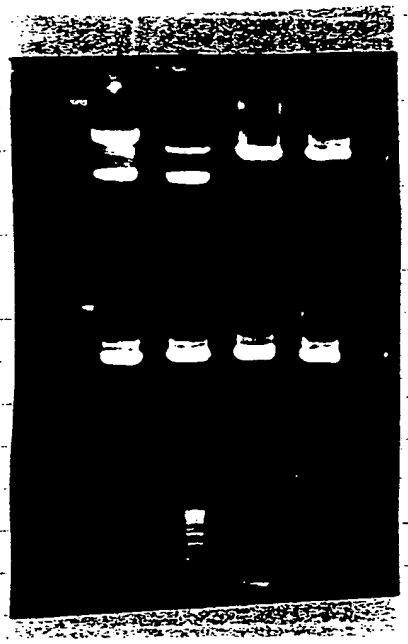
EtOH ppt °/N.

↓

2-21-96 resuspend pllet in 25λ H_2O

Degestion by Enzymes.

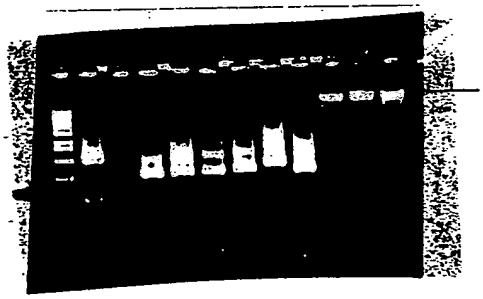
	30λ
DNA + H_2O	25λ
B Buffer	3λ
Enzymes	1λ
" "	1λ



See 2/16/96

37°C incubate 2hrs Smal + Bgl 2's at 25°C incubate

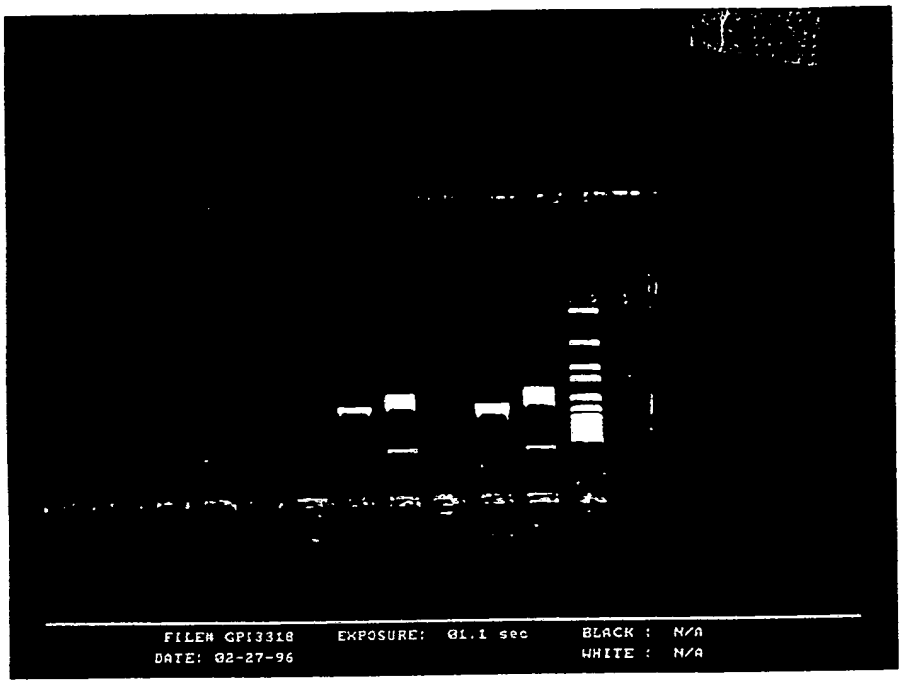
↓ after 2hrs Take 3λ of each sample Runing on the gel:

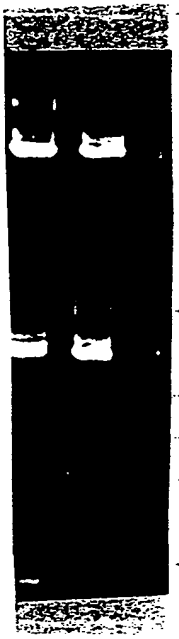


vector

2-20-96 Runing 2% low melting gel: (for construct)

a dark incent. 70c_10

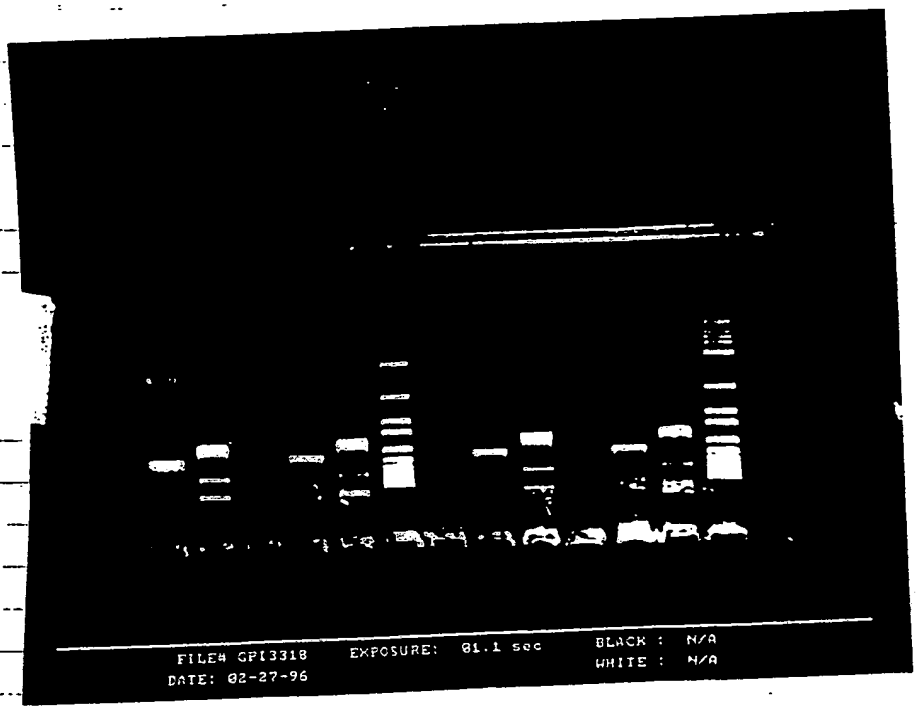




See 2/16/96

5°c incubate

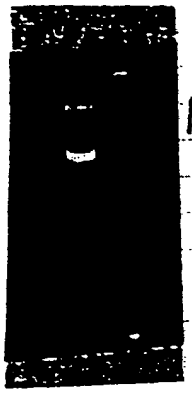
after 2hrs Take 3λ of each sample Runing on the gel:



2-21-96 Degestion pREG vector

cut by BamHI + HindIII.

2/16/96

Take 5ul Running gel ←  pREG BamHI + HindIII.

2-22-96 PCR for constructed See page 125

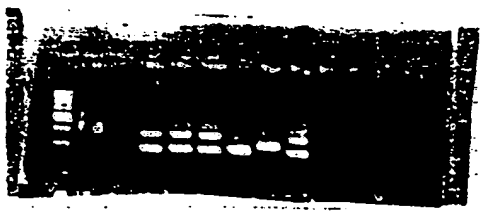
100λ PCR pr.
150λ Promega Buffer (direct purification Buffer)
1ml PCR preps DNA purification Resin)

rt RT incubate 2' pass column

80% isopropanol wash x2

50ul H2O

Take 5ul Running on the gel.



Take 15λ degestion by enzymes:

after degestion: phenol/φ x3 EtOH ppt. resuspend in 10μl H2O

Ligation into vector:

1or	
2λ	5x Ligation Buffer
1λ	Vector
6λ	DNA
1λ	T4 DNA Ligase

RT 5hrs

2-23-96 PCR:

#4 HTTBNb1 — use pA2 3' +5' vector primer

#{5, 6, 7} — use pUC5' +3' vector primer

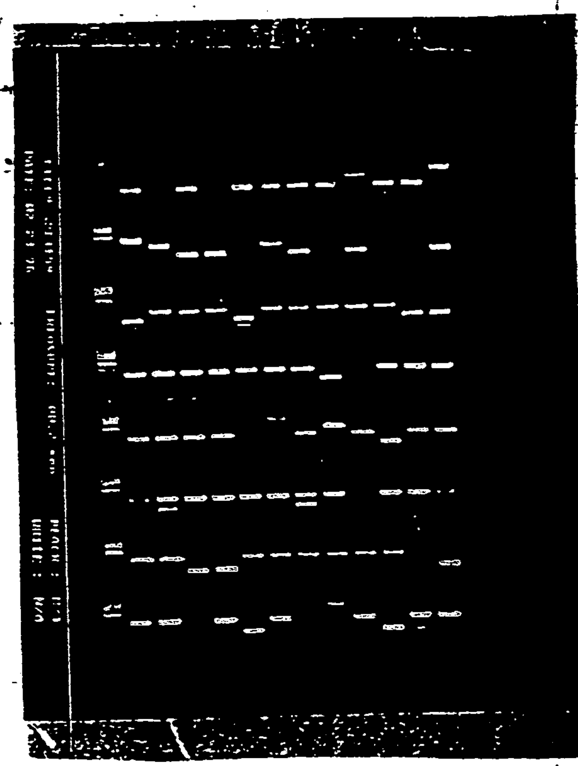
clone:

4: #3 #5 #9 #14

5: #2 #3

6: #1 #2

7: #1 #2



[redacted]-25-96 grow up DNA

2-26-96 make DNA prep:

Sample ID		abs 260.0 nm	abs 280.0 nm		260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
	4-3	0.2317	0.1364	HTTBNb1 A2-GP	1.6990	0.5886	5.8.
	4-5	0.1649	0.0933		1.7679	0.5656	4.1
	4-7	0.0704	0.0403		1.7461	0.5727	1.8.
	4-14	0.0805	0.0427		1.8850	0.5305	2.0.
5	5-2	0.0536	0.0271	HPmucil pos?	1.3729	0.5069	1.3.
6	5-3	0.0877	0.0472		1.8594	0.5378	2.2.
7	6-1	0.0730	0.0381	HTPA	1.9152	0.5221	1.8.
8	6-2	0.0651	0.0339		1.9186	0.5212	1.6.
9	7-1	0.0798	0.0415	HTPA5mg3	1.9239	0.5198	2.0.
10	7-2	0.1008	0.0532		1.8951	0.5277	2.5.
11	6	0.0623	0.0383	465G prim	1.6278	0.6144	1-7/1
12	57	0.0761	0.0474	465T	1.6069	0.6223	1.3.13
13							

Submit for sequencing

HTTBNb1 use pA2 3' +5'

Hpmucil

HTPA

HTPAT

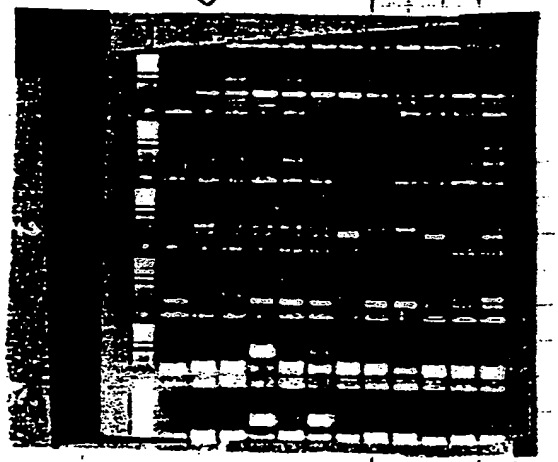
2-26-96 PCR for constructs.

#1 HSABH13 > use pA23'+5'
#3 HOFMB07
#8 HT4 — use pA65'+3'

this new CSG16 NCOL 5' primer.

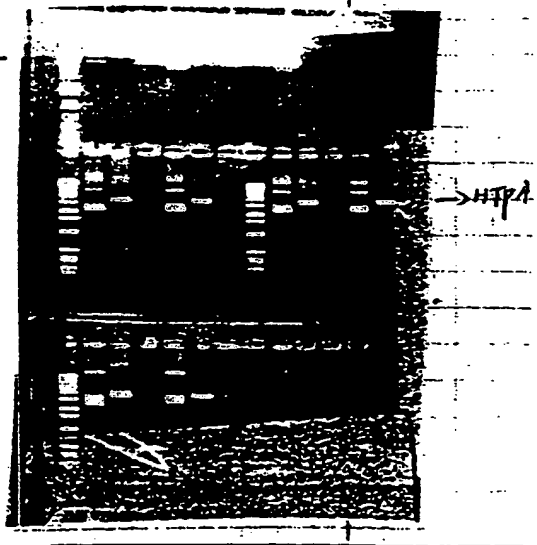
- PCR for CSG16 5'15296 + 14813' NCO1 + HindIII pA260
HTXE133 4293 + 4293
(HNF1016) FASL 4656 + 4657

2-27-96



grow up DNA O/N

- Digestion 2/26/96 DNA prep. Runing 1% agarose gel:



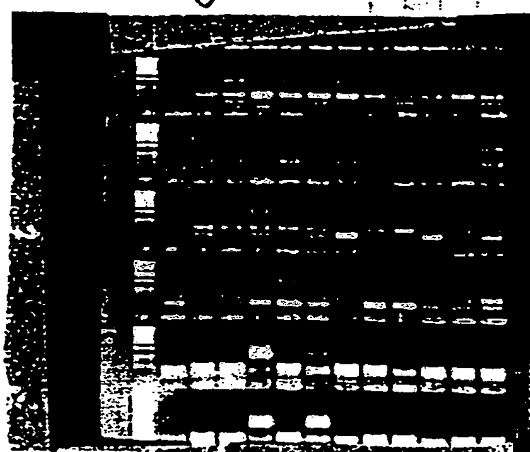
2-26-96 PCR for construct.

#1 HSABH13 > use pA23' + 5'
#3 HOFMB09
#8 HT4 — use pA05' + 3'

this new CSG16 NcoI 5' primer

- PCR for CSG16 5' 1529b + 1481 3' NcoI + HindIII pA2E60
HTXE133 4293 + 4293
(HNF10rb) FASL 4656 + 4657

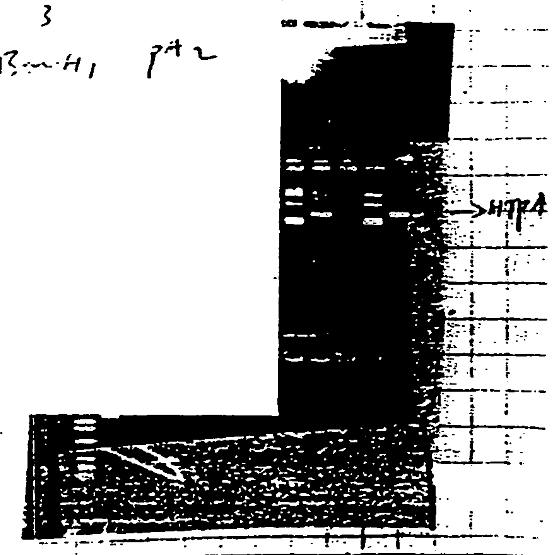
2-27-96



grow up DNA
O/N

- Degestion 2/26/96 DNA prep
5 3
HCACW62 BamHI + BamHI pA2

gel:



2-27-96

12841	pA2 / A2GP 3'
1474	pA2 5'
2785	A2GP 5'

2-28-96

Results file: A:\WORK_RES — Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: One cell
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [No]
Peak Pick: [No] 1:500

Sample ID		abs 250.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1	s	0.3443	0.0233 HSABA13	1.9006	0.5262	1:1
2	u	0.0736	0.0398 HTU	1.8498	0.5406	1:8
3						

Submit for sequencing:

HSABA13 #3 pA2 3' +5'

HTU #4 pA2 3' +5'

2-28-96 (HLHIHC58) TAFReceptor full

Forward
Reverser
HTABLE1 RPR1 RPO2
RPR3 R4
RPO1 R5
F3
R3
F4

Repeat Sequencing

Reverser >add 1% DMSO
F3

2-29-96

2-29-96 PCR for HTXEL33 pAz → use pAz 3'+5'

HTTBN61 A2-GP → use pAz3'+ A2-GP5'

FABL (HNF1α) pRE9 → pRE 3'+5'

CSG16 p026a

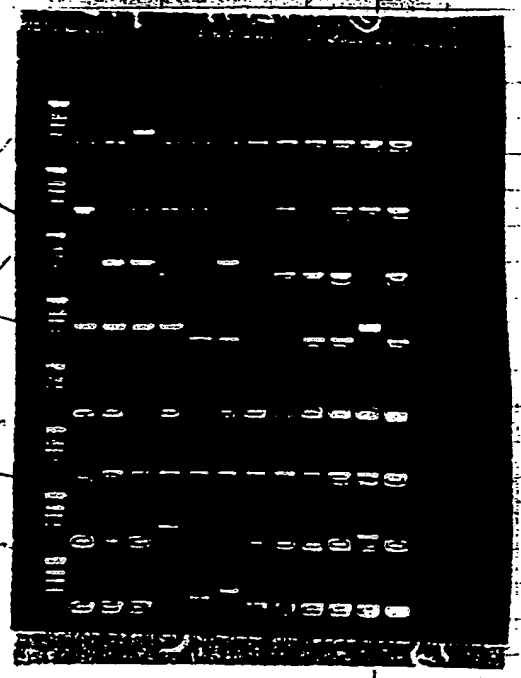
grow up DNA o/n

HTXEL33
pickup #3

HTTBN61
#2 #14

FAL(HNF1α)

CSG16
#4 #16 #18



3/1/96 mini DNA prepr:

Submit for sequencing:

HTXEL33 pAz 3'+5'

HTTBN61 A2-GP 3'+5'

CSG16 pRE3' +3'

Submit HTTBN61 for sequencing

HUMHNF3B: Reverse add 1% DMSO
F3

Nucleic Acid
ReadSamples Method SaveClear Print Q

Results file: A:\WORK_RES Method name: A:\DEFAULT

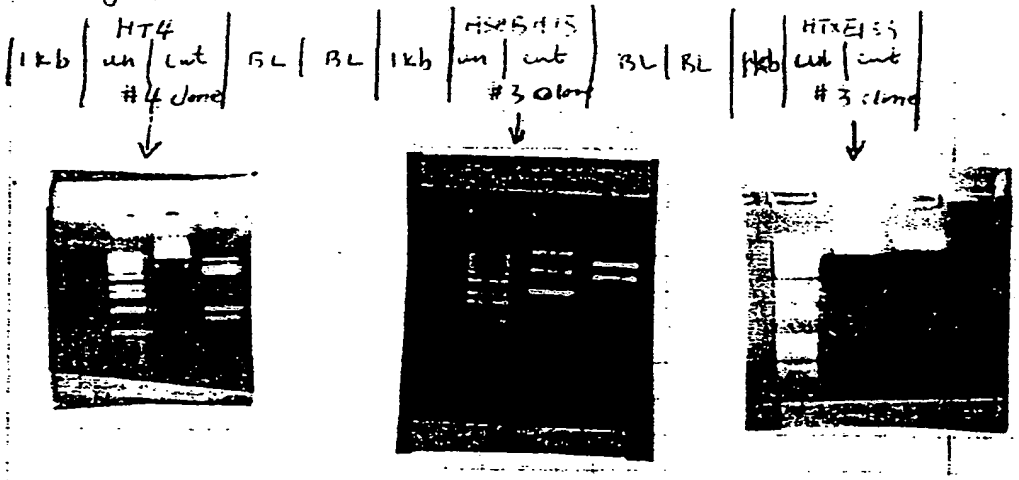
Assay type: General Ratio and Concentration Units: ug/ml
Formula setup: VIEW Background Correction: [No
Sampling device: One cell Concentration: [No]
Read average time: 0.50 sec Peak Pick: [No]

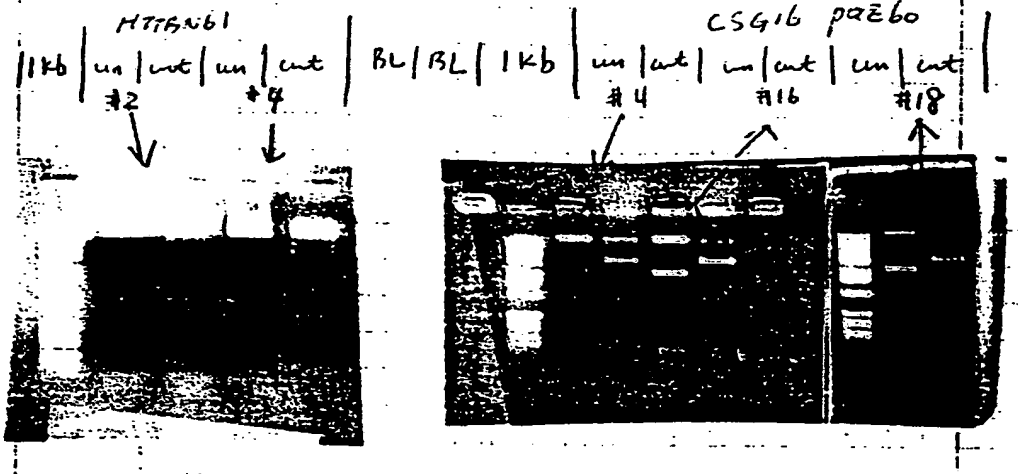
Sample ID	abs 260.0 nm	abs 280.0 nm		260.0 nm / 280.0 nm	260.0 nm / 280.0 nm	1:50
1 HTXEL33	0.0438	0.0234	#3	1.8724	0.5341	1.1
2 HTTBN61	0.0794	0.0412	#2	1.9274	0.5188	2
3	0.0443	0.0245	#14	1.8070	0.5534	1.1
4	0.0171	0.0078	#4	2.1931	0.4560	0.4
5 CSG16	0.0358	0.0184	#16	1.9322	0.5176	0.9
6	0.0244	0.0130	#18	1.8756	0.5332	0.6
7						

5-4-96 Degestion DNA (Seq-ge 151)

				Buffer
(1)	HT4	pAE9	BamHI + HindIII	B
(2)	HSABH13	pAz	BamHI + Asp718	B
(3)	HTXE133	pAz	SamI + Asp718	A
(4)	HTTBN61	Az-GP	BamHI + xbaI	A
(5)	CSG16	pQE60	NcoI + HindIII	B

Running 1% agarose gel :





3-5-96 Digestion for HTTBsiBi/A2-4p #9 clone

cut o/n.

transformation to JM15 cell.

Hp00012 #3 clone

HTPAN03504 #2 clone (short)

HTPAN08504 #2 clone (long)

HTH (from ligation).

pour plate (LB+A+K)

3-6-96 grow up clones at LB+A+K medium 37°C o/n.

(for protein induction)

grow up: Hp00012 #3 1 — 4

HTPA (short) #2 5 — 8

HTPA (long) #2 9 — 12

CSG6 14 — 15

CSG7 16 — 17

3-7-96 take 30λ to 3ml LB+A+K.

37°C 2.5 hrs

add 30λ IPTG to 3ml

37°C 3 hrs

3-7-96 Take 800λ spin down. wash 1

add 15λ of H_2O
15λ 2x SDS loading buffer
mix boil 5'

Running 10% SDS page:

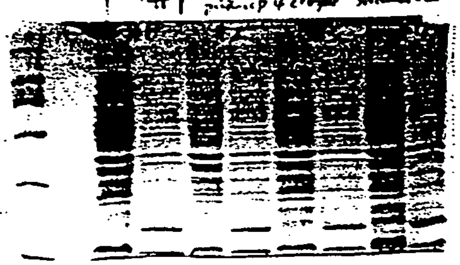
#1 gel: | m | BL | 1 | 1+ | 2 | 2+ | 3 | 3+ | 4 | 4+ |

#2 gel: | m | 5 | 5+ | 6 | 6+ | 7 | 7+ | 8 | 8+ | BL |

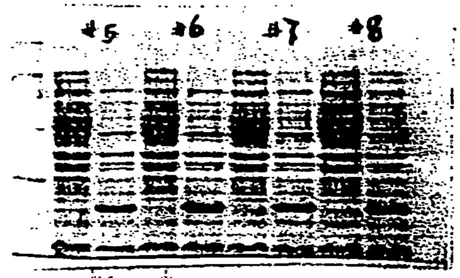
#3 gel: | m | 9 | 9+ | 10 | 10+ | 11 | 11+ | BL | 12 | 12+ |

#4 gel: | m | 14 | 14+ | BL | 15 | 15+ | 16 | 16+ | 17 | 17+ |

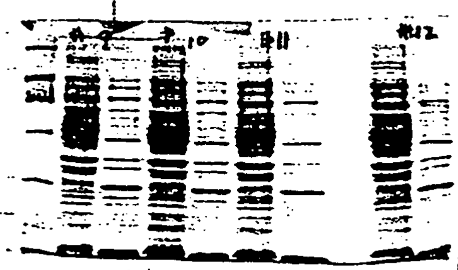
#1 HP0012 #1/transformants pickup 4 clone induction



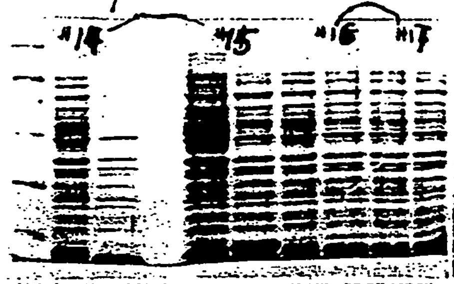
#2 HTPA (short) trail/transformants



HTPA (trail-long) #2/transformants
#3



CS46/p839 #4 CS67/p839

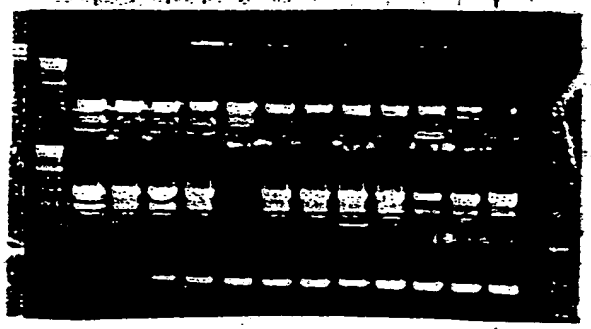


3-8-96 freezing HP0012/p839 BamHI+HindIII #1 2. 3. 4
HTPA (trail short)/p839 BamHI+HindIII # 5. 6. 7 8
HTPA (trail long)/p839 BamHI+HindIII # 9 10 11 12

3-7-96 PCR for HTU (constructs) in wild cell

use 461j + 462v specific primer. Total 36 clone

3-8-96 Running 1% agarose gel:



pickup # 13 #15

(protein induction)

3-9-96 grow up HT4 #13 #15 O/N

LB + A + K medium

3/14/96

→ Jim N: Title HPODV12

HTAA - long #1 clone

HTP4 - short #1

3/15/96

3-11-96 protein induction

↓

Take 30A + 3 mL LB/AMP medium

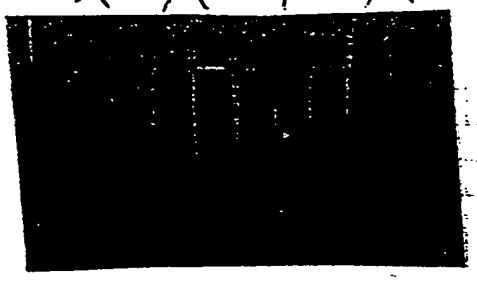
↓ 37°C 2.5 hrs

↓ add 30A of IPTG (~~[redacted]~~)

↓ 37°C 3 hrs

Running 15% SDS page: the cells were not good.

csg16 HTH csg6 csg7 — Repeat 3/7/96 gel



~~Repeat~~

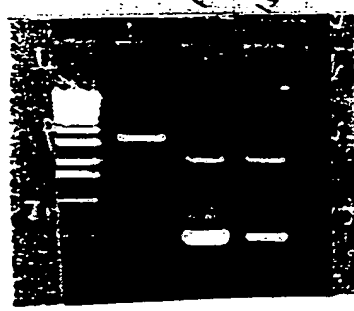
Repeat csg16 HTH ccd2 > construct See 3-11-96

5-11-96 PCR for CSG16 NcoI + BamHI / pA2

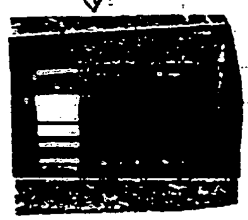
(HCQAJ72) ② CSG16 1529b NcoI 5' + 12121 BamHI 3' (HCQAJ72)
① CSG16 1529b NcoI 5' + 1481 3' HindIII 3' (")

5-12-96 after PCR Take 5λ Running on the gel:

↓ Take 100λ of PCR
100λ promega Buffer (kit)
1 ml PCR DNA Resin
RT 1'
↓ pass column wash X2 80% isp
↓ add 100λ of H2O
↓ Take 5λ Running on the gel



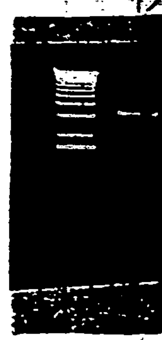
digestion: Take 43λ of PCR insert
5λ of B-Buffer
1λ NcoI
1λ BamHI
37° incubate 2 hrs
loading all sample on the gel:
↓
→ geneclean
↓
after geneclean Running on the gel



after Degestion Running on the gel

cut down insert

Degestion pQE60 NcoI + BamHI
after O/N Take 3λ Running on the gel



pQE60 NcoI + BamHI

the DNA put in Vectastain

3-13-96 HT4 continued:

Digestion BamHI + HindIII

37°c 2hrs

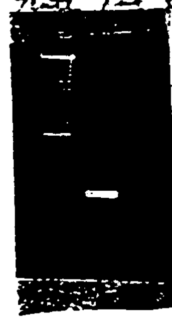
↓ phenol x2

↓ chloro x1

↓ EtOH ppt

↓ resuspend in 10ul H_2O

after PCR purification



— Ligation: to pQE9 vector (BamHI + HindIII)

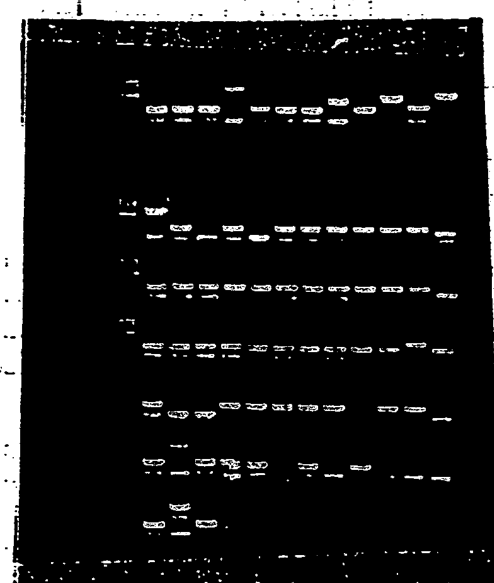
— transformation to M15 cell:

— 37°c o/n

3-14-96 PCR for CSG16/pQE60 (15296+12121) NcoI + BamHI

HT4472/pQE9 BamHI + HindIII

use pQE 3' + 5' primer



CSG16 pickup clone #1 2 3 4

HT4 5.6.7.8

SUPERVISOR- [signature]

DATE- 03/14/96

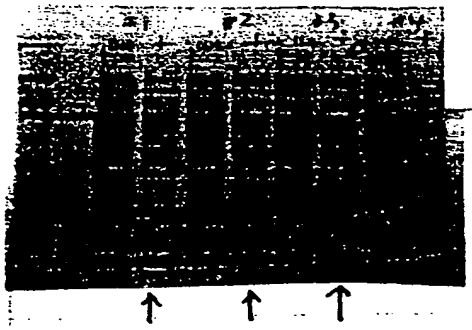
3-15-96 CSG16 protein induction.
HT4

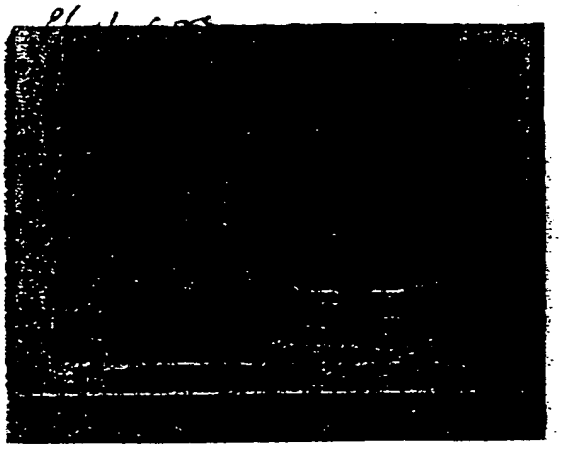
CSG16 1—4 clone
HT4 5—8 clone

#1 gel: |m| BL | 1 | 1+ | 2 | 2+

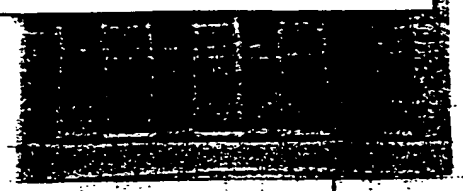
#2 gel: |m| 5 | 5+ | 6 | 6+ | 7 | 7

#3 gel: CSG16/pQE60





clone from Jim Ni 3/26/96



3-15-96 freezing-go CSG16/pQE60 NCo1 + BamH1 #1 #2 #3 clone
HT4ccl72/pQE9 BamH1 + HindIII #5 #6 #7 #8 clone.

3-17-96 grow up CSG16 #1, 2 clone for DNA.
HT4ccl72 #5 #6

3-18-96 Do DNA prep: for CSG16 #1, 2
HT4ccl72 #5, 6

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: One cell
Read average time: 0.50 sec

Units: ug/ml
Background Correction: [No]
Concentration: [No]
Peak pick: [No]

Sample ID		abs 260.0 nm	abs 280.0 nm	1:500	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	
1	CSG16 #1	0.0406	0.0209		1.9434	0.5146	1 ug/ml
2	CSG16 #2	0.0330	0.0176		1.8681	0.5353	0.8
3	HT4 #5	0.0754	0.0406		1.8565	0.5387	1.9 dilute 2 ug/ml
4	HT4 #6	0.0341	0.0161		2.1179	0.4722	0.9
5	pQE60A	0.0656	0.0359		1.8287	0.5468	1.6

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